

1 CLAIMS

- 2
- 3 1. A method of spectrographic measurement, comprising the steps of:
- 4 (a) generating light energy using an excitation source, said light energy being
- 5 caused to fall on a sample to be assayed, causing said sample to output an
- 6 output optical signal;
- 7 (b) generating a plurality of modulation frequencies;
- 8 (c) generating a plurality of heterodyne frequencies to form a set of
- 9 heterodyne signals at said heterodyne frequencies, each of said heterodyne
- 10 frequencies being associated with one of said modulation frequencies;
- 11 (d) coupling said modulation frequencies to said excitation source, causing
- 12 said excitation source to generate excitation energy modulated in intensity in
- 13 proportion to said modulation frequencies;
- 14 (e) sampling a portion of said laser excitation energy to form a reference laser
- 15 excitation signal;
- 16 (f) focusing said output optical signal as an image modulated with said
- 17 plurality of modulation frequencies on an image intensifier;
- 18 (g) intensifying said image to form an intensified image modulated with said
- 19 plurality of modulation frequencies;
- 20 (h) receiving said intensified image modulated with said plurality of
- 21 modulation frequencies on a multielement optical detector;
- 22 (i) generating a plurality of measurement signals using said multielement
- 23 optical detector, each measurement signal associated with a single one of said
- 24 elements;
- 25 (j) for each measurement signal associated with a single one of said elements
- 26 of said multielement optical detector, mixing said measurement signal with
- 27 said heterodyne signal to generate a plurality of low-frequency measurement
- 28 modulation products, one low-frequency measurement modulation product
- 29 being associated with each of said modulation frequencies and comprising

1 the difference between a single modulation frequency and its associated
2 heterodyne frequency and having a measurement amplitude and phase;
3 (k) mixing said reference laser excitation energy with said heterodyne signal
4 to generate a plurality of reference modulation products, one reference
5 modulation product being associated with each of said modulation
6 frequencies and comprising the difference between a single modulation
7 frequency and its associated heterodyne frequency and having a reference
8 amplitude and phase, each of said low-frequency reference modulation
9 products being associated with one of said measurement modulation
10 products;
11 (l) for each of said plurality of low-frequency measurement modulation
12 products, comparing said low-frequency measurement modulation product
13 to its associated low-frequency reference modulation product to generate an
14 output signal indicating characteristics of said sample at the region on said
15 sample associated with each of said elements.

16
17 2. The method of claim 1, wherein said output signal is numerically processed to
18 generate changes over time.

19
20 3. The method of claim 1, wherein said output signal may be graphically displayed.

21
22 4. The method of claim 1, wherein said output signal is numerically processed to
23 generate a desired parameter.

24
25 5. The method of claim 1, wherein said excitation source is a laser.

26
27 6. The method of claim 1, wherein said output optical signal comprises fluorescent
28 energy from said sample

29

- 1 7. The method as in claim 1, wherein said modulation frequencies are harmonically
2 related.
3
- 4 8. The method as in claim 1, wherein excitation source is a laser whose output is
5 modulated by a Pockel's cell.
6
- 7 9. The method as in claim 1, wherein excitation source is a laser whose output is a
8 pulsed laser.
9
- 10 10. The method as in claim 9, wherein said laser is a pulsed-dye laser.
11
- 12 11. The method as in claim 1, wherein excitation source is a light emitting diode.
13
- 14 12. The method as in claim 1, wherein reference modulation products are the low-
15 frequency reference modulation products output during said mixing operation.
16
- 17 13. The method as in claim 1, wherein said comparison is done by measuring the
18 relative phase and amplitude of said low-frequency measurement modulation
19 product as compared to said low-frequency reference modulation product and
20 generating a modulation data point and a phase data point;
21
- 22 14. The method as in claim 13, further comprising:
23 (m) for each element, fitting said modulation data points to a first curve using
24 the method of least squares;
25 (n) for each element fitting said phase data points to a second curve using the
26 method of least squares;
27 (o) comparing said first and second curves to a database to determine
28 characteristics of said sample; and
29 (p) displaying said characteristics.

- 1 15. The method of claim 1, wherein before said excitation energy output by said
2 excitation source is caused to fall on said sample to be measured, the system is
3 calibrated by first using, in place of said sample, a standard consisting of a zero
4 lifetime scattering solution to create a set of normalizing phase and modulation
5 standard values against which said phase and modulation values for said sample
6 are measured.
- 7
- 8 16. A method of spectrographic measurement, comprising the steps of:
- 9 (a) generating light energy using an excitation source, said light energy being
10 caused to fall on a sample to be assayed, causing said sample to output an
11 output optical signal;
- 12 (b) generating a plurality of modulation frequencies;
- 13 (c) generating a plurality of heterodyne frequencies to form a set of
14 heterodyne signals at said heterodyne frequencies, each of said heterodyne
15 frequencies being associated with one of said modulation frequencies;
- 16 (d) coupling said modulation frequencies to said excitation source, causing
17 said excitation source to generate excitation energy modulated in intensity in
18 proportion to said modulation frequencies;
- 19 (e) sampling a portion of said laser excitation energy to form a reference laser
20 excitation signal;
- 21 (f) focusing said output optical signal as an image modulated with said
22 plurality of modulation frequencies on an image intensifier;
- 23 (g) intensifying said image to form an intensified image modulated with said
24 plurality of modulation frequencies;
- 25 (h) receiving said intensified image modulated with said plurality of
26 modulation frequencies on a multielement optical detector;
- 27 (i) generating a plurality of measurement signals using said multielement
28 optical detector, each measurement signal associated with a single one of said
29 elements;

(j) for each measurement signal associated with a single one of said elements of said multielement optical detector, comparing the output of said elements to a standard to generate an output signal indicating characteristics of said sample at the region on said sample associated with each of said elements.

17. Apparatus for performing fluorescence measurement, comprising:

- (a) a light source generating laser excitation energy, oriented to illuminate a sample to be measured and cause said sample to emit fluorescent energy;
- (b) a frequency generator generating a plurality of modulation frequencies and a plurality of heterodyne frequencies, each of said heterodyne frequencies being associated with one of said modulation frequencies said frequency generator being coupled to said excitation source, whereby said source generates excitation energy modulated in intensity in proportion to said modulation frequencies;
- (c) and optical member positioned to receive said laser excitation energy and divert a portion of said laser excitation energy, said portion of said laser excitation energy forming a reference laser excitation signal;
- (d) focusing optics positioned to receive said fluorescent energy and form an image modulated with said plurality of modulation frequencies;
- (e) an image intensifier positioned to receive said image, said image intensifier having an output for outputting an intensified image modulated with said plurality of modulation frequencies;
- (f) a multielement optical detector positioned to receive said intensified image modulated with said plurality of modulation frequencies and generating response thereto a plurality of measurement signals, each associated with a single one of said elements;
- (g) a mixer coupled to receive each of said measurement signals and each of said heterodyne signals and producing in response to said measurement signals and said heterodyne signals a plurality of low-frequency

1 measurement modulation products, one low-frequency measurement
2 modulation product being associated with each of said modulation
3 frequencies and comprising the difference between a single modulation
4 frequency and its associated heterodyne frequency and having a
5 measurement amplitude and phase; and
6 (h) a mixer coupled to said reference laser excitation signals and said
7 heterodyne signals to generate a plurality of low-frequency reference
8 modulation products, one low-frequency reference modulation product being
9 associated with each of said modulation frequencies and comprising the
10 difference between a single modulation frequency and its associated
11 heterodyne frequency and having a reference amplitude and phase, each of
12 said low-frequency reference modulation products being associated with one
13 of said measurement modulation products, each of said low-frequency
14 measurement modulation products, and their associated low-frequency
15 reference modulation products indicating phase and the modulation
16 information.

17
18 18. Apparatus as in claim 17, wherein optical member is a partially silvered mirror.

19
20 19. Apparatus as in claim 17, wherein optical member is a prism.

21
22 20. Apparatus for performing fluorescence measurements, comprising:

23 (a) a light source generating laser excitation energy, oriented to illuminate a
24 sample to be measured and cause said sample to emit fluorescent energy;
25 (b) a frequency generator generating a plurality of modulation frequencies
26 and a plurality of heterodyne frequencies, each of said heterodyne
27 frequencies being associated with one of said modulation frequencies said
28 frequency generator being coupled to said excitation source, whereby said
29 source generates excitation energy modulated in intensity in proportion to

1 said modulation frequencies;
2 (c) and optical member positioned to receive said laser excitation energy and
3 divert a portion of said laser excitation energy, said portion of said laser
4 excitation energy forming a reference laser excitation signal;
5 (d) focusing optics positioned to receive said fluorescent energy and form an
6 image modulated with said plurality of modulation frequencies;
7 (e) an image intensifier positioned to receive said image, said image
8 intensifier having an output for outputting an intensified image modulated
9 with said plurality of modulation frequencies;
10 (f) a multielement optical detector positioned to receive said intensified image
11 modulated with said plurality of modulation frequencies and generating
12 response thereto a plurality of measurement signals, each associated with a
13 single one of said elements; and
14 (g) a calculating device coupled to said measurement signals, said heterodyne
15 signals and said reference laser excitation signals and configured to extract
16 phase and the modulation information.

17
18 21. Apparatus as in claim 20, wherein said calculating device is a computer.

19
20 22. Apparatus as in claim 20, wherein said focusing topics are microscope optics.

21
22 23. Apparatus as in claim 22, wherein said microscope optics are confocal optics.
23 analyzed.

24
25 24. A method of fluorescence measurement, comprising the steps of:

26 (a) generating light energy in the form of laser excitation energy output by an
27 excitation source, said laser excitation energy being caused to fall on a sample
28 to be measured and cause said sample to emit fluorescent energy;
29 (b) generating a plurality of modulation frequencies;

1 (c) generating a plurality of heterodyne frequencies to form a set of
2 heterodyne signals at said heterodyne frequencies, each of said heterodyne
3 frequencies being associated with one of said modulation frequencies;
4 (d) coupling said modulation frequencies to said excitation source causing
5 said source to generate excitation energy modulated in intensity in
6 proportion to said modulation frequencies;
7 (e) sampling a portion of said laser excitation energy to form a reference laser
8 excitation signal;
9 (f) focusing said fluorescent energy as an image modulated with said
10 plurality of modulation frequencies on an image intensifier;
11 (g) intensifying said image to form an intensified image modulated with said
12 plurality of modulation frequencies;
13 (h) receiving said intensified image modulated with said plurality of
14 modulation frequencies on a multielement optical detector;
15 (i) generating a plurality of measurement signals using said multielement
16 optical detector, a single signal being output from each of the elements of said
17 multielement optical detector, each measurement signal associated with a
18 single one of said elements;
19 (j) for each measurement signal associated with a single one of said elements
20 of said multielement optical detector, mixing said measurement signal with
21 said heterodyne signal to generate a plurality of low-frequency measurement
22 modulation products, one low-frequency measurement modulation product
23 being associated with each of said modulation frequencies and comprising
24 the difference between a single modulation frequency and its associated
25 heterodyne frequency and having a measurement amplitude and phase;
26 (k) mixing said reference laser excitation signal with said heterodyne signal to
27 generate a plurality of low-frequency reference modulation products, one
28 low-frequency reference modulation product being associated with each of
29 said modulation frequencies and comprising the difference between a single

1 modulation frequency and its associated heterodyne frequency and having a
2 reference amplitude and phase, each of said low-frequency reference
3 modulation products being associated with one of said measurement
4 modulation products;
5 (l) for each of said plurality of low-frequency measurement modulation
6 products, comparing said low-frequency measurement modulation product
7 to its associated low-frequency reference modulation product to measure the
8 relative phase and amplitude of said low-frequency measurement
9 modulation product as compared to said low-frequency reference modulation
10 product and generating a modulation data point and a phase data point;
11 (m) for each element, fitting said modulation data points to a first curve using
12 the method of least squares;
13 (n) for each element fitting said phase data points to a second curve using the
14 method of least squares;
15 (o) comparing said first and second curves to a database to determine
16 characteristics of said sample; and
17 (p) displaying said characteristics.

18
19 25. The method of claim 24, wherein before said excitation energy output by said
20 excitation source is caused to fall on said sample to be measured, the system is
21 calibrated by first using, in place of said sample, a standard consisting of a zero
22 lifetime scattering solution to create a set of normalizing phase and modulation
23 standard values against which said phase and modulation values for said sample
24 are measured.

25
26 26. The method of claim 25, wherein said normalizing phase and modulation
27 standard values are generated by the steps of:

28 (q) causing said generated light energy in the form of laser excitation energy
29 output by said excitation source, said laser excitation energy being caused to

1 fall on a zero lifetime standard, causing said sample to output a reference
2 standard optical signal;
3 (r) generating a plurality of modulation frequencies;
4 (s) generating a plurality of heterodyne frequencies to form a a set of
5 heterodyne signals at said heterodyne frequencies, each of said heterodyne
6 frequencies being associated with one of said modulation frequencies;
7 (t) coupling said modulation frequencies to said excitation source causing
8 said source to generate excitation energy modulated in intensity in
9 proportion to said modulation frequencies;
10 (u) sampling a portion of said laser excitation energy to form a reference laser
11 excitation signal;
12 (v) focusing said reference standard optical signal as a standard image
13 modulated with said plurality of modulation frequencies on said image
14 intensifier;
15 (w) intensifying said standard image to form an intensified standard image
16 modulated with said plurality of modulation frequencies;
17 (x) receiving said intensified standard image modulated with said plurality of
18 modulation frequencies on said multielement optical detector;
19 (y) generating a plurality of measurement signals using said multielement
20 optical detector, a single signal being output from each of the elements of said
21 multielement optical detector, each measurement signal associated with a
22 single one of said elements;
23 (z) for each measurement signal associated with a single one of said elements
24 of said multielement optical detector, mixing said measurement signal with
25 said heterodyne signal to generate a plurality of low-frequency measurement
26 modulation products, one low-frequency measurement modulation product
27 being associated with each of said modulation frequencies and comprising
28 the difference between a single modulation frequency and its associated
29 heterodyne frequency and having a measurement amplitude and phase;

1 (aa) mixing said reference laser excitation signal with said heterodyne signal
2 to generate a plurality of low-frequency reference modulation products, one
3 low-frequency reference modulation product being associated with each of
4 said modulation frequencies and comprising the difference between a single
5 modulation frequency and its associated heterodyne frequency and having a
6 reference amplitude and phase, each of said low-frequency reference
7 modulation products being associated with one of said measurement
8 modulation products;
9 (bb) for each of said plurality of low-frequency measurement modulation products,
10 comparing said low-frequency measurement modulation product to its associated
11 low-frequency reference modulation product to measure the relative phase and
12 amplitude of said low-frequency measurement modulation product as compared to
13 said low-frequency reference modulation product and generating a reference
14 standard modulation data point and a reference standard phase data point.